Rejection under 35 U.S.C. §112, first paragraph

Claim 5 is rejected under 35 U.S.C. §112, first paragraph, for lack of written description. Specifically, the Examiner argues that the specification lacks a description which would enable one skilled in the art to make any "peptide" or any "polymer" of Claim 5. Applicants respectfully disagree.

Applicants submit that it is well known in the art how to cap a tripeptide of the present invention with any "peptide" or any "polymer". Specifically, Applicants teach general chemical synthesis methods for tripeptides and peptides (see page 6, lines 34 through page 11, line 29). Using the methodology taught therein, it would be possible to cap the amino-terminal amino acid with an additional amino acid(s) to create a peptide.
Likewise, the tripeptides of the present invention could also be readily capped with a polymer (using either the available termini or side chain functional groups). This is described in the specification on page 25, lines 18-20: "The tripeptides can be covalently attached by surface grafting, co-polymerization, non-covalent incorporation into a matrix or otherwise encapsulating as biomedical materials."

The methodology required to achieve this type of attachment between tripeptide and polymer is well described in various generally available references and is well known to persons skilled in the art. For example, (while protecting the side chain functionality of the tripeptide) the main chain functional groups on the termini of the peptide can be used to:
(1) Create an ester linkage to the C-terminus. For example, one can make a mixed anhydride of the peptide, couple hydroxyl-bearing polymers (e.g., polyvinyl alcohol, polylactic acid, hydroxy-terminated polyethylene glycol) and deprotect side chain protecting groups with acid (i.e., tert-butyl for serine hydroxyls and trityl for asparagine and glutamine amides). (See, as a reference for synthesis of esters via a mixed anhydride: Kim, S. et al., *Tetrahedron Lett.* 24:3365 (1983)). Other coupling conditions that would work for this type of esterification include the use of carbodiimide reagents (e.g., dicyclohexyl-carbodiimide (DCC) in the presence of DMAP (Hassner, A. and V. Alexanian, *Tetrahedron Lett.*, 19(46): 4475 (1978)); alternatively, one could use acidolysis of sidechain protecting groups using tBu or trityl (see Eder, U. et al., *Chem. Ber.*, 110:3161 (1977); Choy, Y.M. and A.M. Unrau, *Carbohydr. Res.* 17:439 (1971), respectively).
(2) Create an amide linkage to the C-terminus. For example, one can use DCC for coupling of amino-bearing polymers (e.g., polyallylamine). This would

generate an amide linkage of the tripeptide to the polymer - one which is generally less susceptible (i.e., longer lived) than the corresponding esters towards *in vivo* metabolic hydrolysis. (See Sheehan, J.C. and G.P.J. Hess (*Am. Chem. Soc.* 77:1067 (1955)) for methods of making amides via DCC coupling of amine and a carboxylic acid).

(3) Create an amide linkage to the N-terminus. For example, one can couple the peptide's amino terminus with an acylating agent (e.g., the mixed anhydride of a carboxyl-bearing polymer (e.g., polymethacrylic acid or co-polymers), using the methodology of Kim et al., *supra*.

Alternatively, the main chain termini of the tripeptide can be protected while the side chain functional groups are used to:

(1) Create an ester linkage to side chain hydroxyls. For example, one could couple the side chain hydroxyl groups of serine to carboxyl-bearing polymers (e.g., polymethacrylic acid or co-polymers) via a mixed anhydride (Kim et al., *supra*);

(2) Create an imide linkage to side chain amides. For example, one could couple the side chain amide of asparagine or glutamine to carboxyl-bearing polymers (e.g., polymethacrylic acid or co-polymers) via carbodiimide coupling (Hassner and Alexania, *supra*) or by use of a mixed anhydride (Kim et al., *supra*).

Although the above discussion relates to ester, amide, and imide linkages between the tripeptides of the present invention and polymers, one skilled in the art would readily be able to extend the concepts described from carbonyl-based linkages to sulfonyl-based linkages, as a mechanism for linkage between the present tripeptides and polymers. Thus, the methodology can also be applied to sulfonates, sulfonamides, and sulfonimides as required.

In view of the foregoing remarks and amendment to Claim 5, Applicants respectfully request withdrawal of the rejection. Additionally, Applicants request that the above discussion be considered in light of new Claim 23.

Rejection under 35 U.S.C. §112, second paragraph

Claims 14 and 21 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Specifically, the Examiner states that the phrase "selected from the group consisting of... trachoma, or Osler-Webber-Rendu disease" in Claim 14 is improper Markush

group language; and that the language "combinations thereof" in Claim 21 is vague.

Claim 14 has been amended to replace the word "or" with the word "and" in order to change the claim to proper Markush group language. Claim 21 has been amended to delete the words "combinations thereof" from the claim. In light of these amendments, Applicants respectfully request withdrawal of the rejection.

Applicants have also made minor amendments in punctuation to Claims 1, 2, 3 and 5, and added the word "and" to Claim 3 to correct claim language terminology. Applicants respectfully submit that all such amendments have been entered solely to more clearly articulate the claimed invention.

Claim Objections

Claims 4-6 are objected to under 37 C.F.R. §1.75(c) as being of improper dependent form for failing to further limit the subject matter of previous claims. Claims 4-6, which are dependent on Claim 1, enlarge the scope of Claim 1 by claiming a tripeptide which is capped with an additional peptide, thus creating a polypeptide which is of greater scope than a tripeptide.

Applicants have amended the claims. Specifically, the terms "peptide" and "polymer" have been removed from Claim 5. As such, none of the additional limitations of Claims 4-6 seek to enlarge the scope of Claim 1 beyond a tripeptide. Capping the tripeptide (at the amino-terminal or carboxy-terminal end) does not create a polypeptide. In light of the amendments, Applicants respectfully request withdrawal of the rejection.

Rejection under 35 U.S.C. §102(b)

Claims 1, 4 and 7 are rejected under 35 U.S.C. §102(b) as being anticipated by Malle et al (*Arteriosclerosis and Thrombosis*, 14(3): 345-351 (1994)). Malle et al teach the RDG tripeptide as a consensus sequence present in a variety of adhesive plasma proteins of the fibrinolytic system, RGD as capped in apolipoprotein(a) [apo(a)], and a composition containing RGD and l-lipoprotein(1). The Examiner states that since angiogenesis-inhibitory characteristics are inherent in the tripeptide RGD, this reference anticipates Claims 1, 4, and 7. Applicants have amended the claims and respectfully traverse the Examiner's rejection. Applicants' respectfully request withdrawal of the rejection in view of the amendments herein.

Rejection under 35 U.S.C. §102(a)

Claims 1-4, 7 and 8 stand rejected under 35 U.S.C. §102(a) as being anticipated by Maeshima et al (*J. Biol. Chem.* 275(28): 21340-21348 (2000)). The Examiner notes that Maeshima et al teach: (1) that the SNS tripeptide is anti-angiogenic; (2) that SNS is found in the α3 chain of Type IV collagen and is capped; and (3) a collagen composition containing the SNS sequence in an angiogenesis-inhibitory amount. Applicants respectfully traverse this rejection. Maeshima et al identify the anti-angiogenic capacity of the noncollagenous 1 (NC1) domain of the α3 chain of type IV collagen using several *in vitro* and *in vivo* assays. However, Applicants note that the minimum polypeptide sequence comprising the SNS tripeptide sequence studied in the work of Maeshima et al consisted of the tum-4 mutant (comprising 64 amino acids of the C-terminus of tumsatin (see page 21346, column 2, lines 3-4)). Further, the authors specifically state that "although the isolated peptide 185-203 [including the SNS sequence] and tum-4 mutant containing this sequence inhibit melanoma cell proliferaction and bind the $\alpha v\beta 3$ receptor, it is **not** responsible for the anti-angiogenic activity of tumstatin" (page 21347, column 2, paragraph 1; emphasis added). Furthermore, the authors conclude that "the exclusive anti-angiogenic activity [of tumstatin] is contained within amino acids 54-132" (page 21347, column 2, paragraph 2). In contrast, the SNS sequence of Applicants' invention is located within amino acids 189-191. Therefore, Maeshima et al in fact teach away from Applicants' SNS tripeptide. In light of the clear conclusions stated by Maeshima et al, Applicants submit that that the Examiner has incorrectly concluded that Maeshima et al teach that Applicants' isolated SNS tripeptide is anti-angiogenic.

Furthermore, for sake of argument, even if one assumed that Maeshima et al's comments on page 21347, paragraph 2 suggested that SNS within the α3(IV) NC1 domain was anti-angiogenic, the theoretical disclosure of a collagen composition comprising a full-length anti-angiogenic polypeptide (which contains the SNS sequence) would not enable a person of skill in the art to conclude that the isolated SNS tripeptide would itself necessarily have anti-angiogenic activity.

As further support for Applicants' position, Applicants respectfully direct the Examiner's attention to a series of references that clearly demonstrate that an isolated peptide fragment can have very different biological properties (i.e., activity) than the native protein from which that isolated peptide fragment is derived.

First, a contiguous peptide sequence isolated from a larger polypeptide can be less active than the polypeptide from whence it was isolated. For example, tendamistat (isolated from culture fluids of *Streptomyces tendae* 4158 (ATCC# 31210)) is a very potent (K_i = 0.2 nm) 74 residue protein inhibitor of porcine pancreatic α-amylase. The active sequence of tendamistat is the tetrapeptide Ser17-Trp18-Arg19-Tyr20. However, it has been determined that both linear and cyclic versions of the active sequence are much less active (orders of magnitude) than the full-length native protein. See, Matter, H. and H. Kessler, *J. Amer. Chem. Soc.* 117(12): 3347-59 (1995), wherein the authors state "... the suggested active tetrapeptide sequence alone is not responsible for the strong binding between tendamistat and α-amylase...". Further, Hirano, T. et al. (*Peptide Chemistry*, 34:273-276 (1996)) report that two short cyclic peptides (i.e., Ten(16-22) and Ten(15-23)) having the inhibitory site (Trp18-Arg19-Tyr20) of α-amylase inhibitor tendamistat only inhibited pancreatic α-amylase weakly, while a third peptide (i.e., Ten(14-24)) showed no inhibition.

Alternatively, a contiguous peptide sequence isolated from a larger polypeptide can be more active than the polypeptide from whence it was isolated. For example, the protein osteopontin--a noncollagenous bone extracellular matrix protein that is a secreted adhesive glycoprotein of about 41.5 kD with a functional 'RGD' cell-binding domain that interacts with the αvβ3 cell surface integrin heterodimeric receptor.

Takahashi, K. et al. (*Biochem. Mol. Biol. Internat.* 46(6): 1081-1092 (1998)) report that "the N-terminal fragment containing the RGD motif [of osteopontin] enhanced the adhesion of mouse and human fibroblasts by 2.9- and 2.8-fold in comparison with full-length osteopontin, respectively. The enhanced adhesion of both cells mediated by the N-terminal fragment was significantly suppressed by addition of the C-terminal fragment lacking the RGD motif ..." Subsequent work by Lehenkari, P.P. and M.A. Horton (*Biochemical and Biophysical Research Communications* 259(3): 645-650 (1999)) reports atomic force microscopy measurements of integrin binding forces for several short linear RGD hexapeptides and larger peptides and proteins containing the RGD sequence. The authors determined that, in contrast to short linear RGD hexapeptides, larger peptides and proteins containing the RGD sequence (e.g., osteopontin and echistatin) showed different binding affinities. Thus, this demonstrated "that the context of the RGD sequence within a protein has considerable influence upon the final binding force for receptor interaction".

Thus, Applicants contend that one cannot predict the biological properties of an isolated tripeptide, when one has knowledge of the biological properties of the full-length polypeptide. Therefore, a disclosure which teaches a specific polypeptide containing the SNS tripeptide fails to anticipate the present invention, wherein the angiogenesis-inhibitory properties of the isolated SNS tripeptide are taught.

In light of the above discussion, including the clear negative teaching of the cited art, Applicants respectfully request withdrawal of the rejection.

Rejection under 35 U.S.C. §103

Claims 1 and 4-22 are rejected under 35 U.S.C. §103(a) as being obvious over Buckley et al. (*Nature* 397: 534-539 (11 Feb 1999)) in view of Matsuo et al. (U.S. Patent No. 5,187,156).

The teachings of Buckley et al show that a RGD tripeptide has anti-angiogenic properties and that the tripeptide is contained within a larger protein and is capped. From the teachings of Blaschuk et al (U.S. Patent No. 6,169,071 B1), the Examiner states that it is well known in the art that the tripeptide may be capped at the amino-terminal with an acetyl group and may be capped at the carboxy-terminal with an amide group. Buckley et al do not teach the administering of an effective anti-angiogenic amount of the tripeptide in a pharmaceutically acceptable carrier. Matsuo et al teach combining the tripeptide DTrp-Phe with therapeutic properties with a pharmaceutically acceptable carrier for therapeutic use in the treatment and prevention of asthma.

The Examiner argues that it would have been obvious to one of ordinary skill in the art to (i) combine the tripeptide RGD with anti-angiogenic properties with a pharmaceutically acceptable carrier and to administer the combination by any well-known delivery method for therapeutic use in the treatment of angiogenesis; and (ii) administer the tripeptide and carrier to treat the symptoms of angiogenesis.

Applicants have amended Claim 1 to delete the RGD tripeptide from the scope of the claim. In light of the amendment, the Buckley et al reference is no longer applicable and Applicants respectfully request withdrawal of the rejection.

Claims 1, 9 and 12-19 are rejected under 35 U.S.C. §103(a) as being obvious over Buckley et al. (*supra*) in view of Wickham et al. *(J. Virology* 71(11): 8221-8229 (Nov. 1997)).

The teachings of Buckley et al is set forth above. However, Buckley et al do not teach administering the RGD tripeptide by encoding nucleic acid and incorporating this into a vector, adenovirus, or DNA. Wickman et al. teach a method of incorporating the RGD angiogenesis-inhibitory peptide sequence into an adenovirus and nucleic acids.

The Examiner argues that it would have been obvious to one of ordinary skill in the art at the time of Applicants' invention to administer the claimed angiogenesis-inhibitory peptide to tissue via encoding nucleic acid and incorporation into a vector, adenovirus, or DNA. In light of the amendment to Claim 1, wherein the RGD tripeptide is no longer included within the scope of the claim, Applicants' submit that the cited references do not make the present invention obvious and respectfully request the Examiner to withdraw this rejection and reconsider the claims as amended.

Respectfully submitted,

INNA Y. BELOPOLSKY
ATTORNEY FOR APPLICANTS
REGISTRATION NO. 43,319
TELEPHONE: 302-992-4406
FACSIMILE: 302-992-5374

Dated: [illegible]

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In showing the changes below, deleted material is braketed, inserted material is underlined.

In The Claims:

Please amend the claims as follows:

1. (Amended One Time) An angiogenesis-inhibitory tripeptide of formula aa1-aa2-aa3, having a first amino acid (aa1), a second amino acid (aa2) and a third amino acid (aa3), wherein:[,]

(a) said first amino acid is selected from the group consisting of Ser, Thr, Ala, Phe, Tyr, Cys, Gly, Leu, Lys, Pro, Arg, Gln, Glu, Asp, Asn, His, Met, Ile, Trp, Val, diaminoproprionic acid and *trans*-4-hydroxy-proline;

(b) said second amino acid is selected from the group consisting of Asn, Ala, Gly, Asp, Glu, Gln diaminoproprionic acid and *trans*-4-hydroxy-proline; and

(c) said third amino acid is selected from the group consisting of Ser, Thr, Ala, Phe, Tyr, Cys, Gly, Leu, Lys, Pro, Arg, Gln, Glu, Asp, Asn, H[h]is, M[m]et, Ile, Trp, Val, diaminoproprionic acid and *trans*-4-hydroxy-proline;

and wherein the tripeptide is not Arg-Gly-Asp.

2. (Amended One Time) The angiogenesis-inhibitory tripeptide of Claim 1, wherein:

(a) said first amino acid is selected from the group consisting of Ser, Thr, Cys, and diaminoproprionic acid;

(b) said second amino acid is selected from the group consisting of Asn and Gln; and

(c) said third amino acid is selected from the group consisting of Ser, Thr, Cys, and diaminoproprionic acid.

3. (Amended One Time) The angiogenesis-inhibitory tripeptide of Claim 1, wherein:[,]

(a) said first amino acid is Ser;

(b) said second amino acid is Asn or Gln; and

(c) said third amino acid is Ser.

5. (Amended One Time)_ The angiogenesis-inhibitory tripeptide of Claim 1, wherein the first amino acid is an amino-terminal and the third amino acid is a carboxy-terminal, wherein:[,]

(a) the amino-terminal is capped with a compound selected from the group consisting of acetyl, benzoyl, alkylsulfonyl, arylsulfonyl, alkylaminoacyl, arylaminoacyl, and formyl[, peptide and polymer]; and

(b) the carboxy-terminal is capped with a compound selected from the group consisting of NH_2, OH, and NHR, wherein R is selected from the group consisting of alkyl and aryl.

6. (Amended One Time)_ The angiogenesis-inhibitory tripeptide of Claim 5[4], wherein the amino-terminal is capped with an acetyl group and the carboxy-terminal is capped with an NH_2[amide] group.

14. (Amended One Time) The method of Claim 9, wherein the angiogenesis is associated with a condition selected from the group consisting of ocular neovascular diseases, choroidal neovascular diseases, retina neovascular diseases, neovascularization of the angle, Bartonellosis, chronic inflammation, osteoarthritis, rheumatoid arthritis, atherosclerosis phemphigoid, trachoma, and[or] Osler-Webber-Rendu disease.

21. (Amended One Time) The method of Claim 11, wherein said tripeptide is administered in conjunction with a therapeutic compound, the therapeutic compound being selected from the group consisting of chemotherapeutics, antibiotics, antivirals, anti-inflammatories, targeting compounds, cytokines, immunotoxins, anti-tumor antibodies, angiogenic inhibitors, anti-edema agents, and radiosensitizers [and combinations thereof].

Please add new Claim 23 as follows:

23. (New Claim) An angiogenesis-inhibitory compound, comprising a capped tripeptide of formula aa1-aa2-aa3, having a first amino acid (aa1), a second amino acid (aa2) and a third amino acid (aa3), wherein:

(d) said first amino acid is selected from the group consisting of Ser, Thr, Ala, Phe, Tyr, Cys, Gly, Leu, Lys, Pro, Arg, Gln, Glu, Asp, Asn, His, Met, Ile, Trp, Val, diaminoproprionic acid and

trans-4-hydroxy-proline and wherein said first amino acid is capped with a compound selected from the group consisting of peptide and polymer;

(e) said second amino acid is selected from the group consisting of Asn, Ala, Gly, Asp, Glu, Gln diaminoproprionic acid and *trans*-4-hydroxy-proline; and

(f) said third amino acid is selected from the group consisting of Ser, Thr, Ala, Phe, Tyr, Cys, Gly, Leu, Lys, Pro, Arg, Gln, Glu, Asp, Asn, His, Met, Ile, Trp, Val, diaminoproprionic acid and *trans*-4-hydroxy-proline and wherein said third amino acid is capped with a compound selected from the group consisting of NH_2, OH, and NHR, wherein R is selected from the group consisting of alkyl and aryl;

and wherein the tripeptide is not Arg-Gly-Asp.